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JUL 25 1997

CALFED Bay-Delta Program Office  
1416 Ninth Street, Suite 1155  
Sacramento CA 95814

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\$108,591.50

Research Proposal Entitled  
**"The Genotoxic and Genetic Effects of Pesticides on River Otters in the  
Sacramento-San Joaquin Delta of California"**  
RFP: 1997 Category III Ecosystem Restoration Projects and Programs  
Principal Investigator - **Michael L. Johnson**

Dear Colleague:

It is our pleasure to present for your consideration the referenced proposal in response to **the CALFED Bay-Delta Program RFP**.

Please call on the principal investigator for scientific information. Administrative questions may be directed to me or my assistant, René Domino, at the above address and phone number. We request that correspondence pertaining to this proposal and a subsequent award be sent to the Office of Research and to the principal investigator.

Sincerely,

A handwritten signature in cursive script that reads "Sandra M. Dowdy".  
Sandra M. Dowdy  
Contracts and Grants Analyst

Enclosure

cc: M. Johnson

## **I. Executive Summary**

- a.** Title: The Genotoxic and Genetic Effects of Pesticides on River Otters in the Sacramento-San Joaquin Delta of California, Dr. Michael Johnson.
- b.** The proposed research addresses the effects of pesticides applied within a California ecosystem, in particular, on the North American river otter, *Lutra canadensis* (Schreber 1776). It attempts to further the understanding of mechanisms and processes to advance the predictive power of theory in ecotoxicology as well as conservation. River otters will be studied within the Sacramento-San Joaquin Delta in areas of heavy pesticide inputs. These populations will be compared to otters living in nearby watersheds within valley habitats, that do not receive significant pesticide contamination. The project includes a biomarker investigation to quantify exposure to and short-term effects of as well as a genetic investigation to estimate long-term effects of important pesticides on river otters in the Delta.

The primary biological objectives are addressed by the following four specific questions. Will biomarker responses among river otters exposed to different concentrations of pesticides vary with dose? Will biomarker responses permit a link of exposure to direct damage to individuals? Will genetic differences, if found, be consistent in river otters across field replicates? And finally, can biomarker responses complement population-genetic patterns when building a spatial understanding of bioavailable contaminants?

- c.** The following phases represent divisions of the project by task-type, funding, and scheduling. All phases, are described here. This proposal seeks funding only for Phase III. Sampling locations are currently being selected as Phase I. In a preliminary distribution assessment of pesticides and otters, databases from local agencies and field surveys are compiled to select experimental and reference locations for comparison. Crayfish and possibly otter spraint (fecal) samples from selected locations will be sampled for chemical analysis to confirm the site characterization that was derived from database information. In Phase II, otter spraints will be systematically collected from sample locations and analyzed by molecular genetic techniques. Microsatellite DNA variation will provide information about the spatial distribution of otters, and will permit analysis of genetic differences among populations that will later be statistically analyzed to test for correlations to exposure parameters. In Phase III, otters will be trapped for collection of tissue samples for biomarker analysis of exposure and genotoxic damage. In addition, the health of the animals will be assessed, animals will be marked, and released. Finally, in Phase IV, samples drawn from otters during Phase III will be analyzed in the laboratory. Serum will be analyzed for acetylcholinesterase levels to ascertain exposure to acetylcholinesterase inhibitors. DNA strand breaks will be measured in blood. Values derived from these analyses will be critical to the final data analysis.

- d.** Trapping is the most labor-intensive and time-extensive portion of the project as trapping of each animal requires the setting of many traps over many days. It is a critical step, however, to linking pesticide exposure to measures of short term damage, and ultimately to genetic change, a measure of long term effect. The Delta contains pesticide loads sufficient to be toxic to invertebrates and fishes and these chemicals have been implicated in the decline of other species such as striped bass. Because of its trophic position, otters are likely to bioaccumulate pesticides that are consumed or absorbed by species lower in the food chain. The proposed work on river otters is consistent with the goals of several regional programs. A report prepared for the Bay/Delta oversight council emphasizes that many contaminants reach toxic concentrations throughout the Delta and that while it is not possible to set standards for every affected species, it

is critical to consider the most sensitive and indicative species, such as top mammalian carnivores in which "effects of accumulated contaminants will be most apparent (p 103)." The CALFED Bay Delta Program Overview (1996) points repeatedly to the goal of sustaining and restoring ecosystem health, including restoration of conditions to those that permit long-term viability and integrity of key wildlife species at all ecological levels.

e. Field assistance will be required during much of the trapping program in order to have the necessary logistic support to put out traps, operate the boat, and process animals. This proposal requests an assistant at the level of PGRI for 50% time for three years (exact figures are shown on Table 1). Funds are also requested for the purchase of a 10 HP outboard motor (\$1600) to be used on a small boat available on loan. Travel funds are requested to defray some of the costs of the use of a personal vehicle for boat and supply transport (\$500 per year). Funds are requested at this time for the purchase of 30 traps (\$250 each = \$7500). An additional 4 traps (\$1000) are expected to be purchased during the subsequent years of the project to replace damaged or lost traps from the first year of the project. Finally, funds are requested for the purchase of anesthetic drugs and supplies for the processing of animals upon capture (\$250 per year). This project is anticipated to have no immediate third party impacts. No action levels and no direct mitigation procedures are proposed.

f. Dr. Michael Johnson is an Associate Research Engineer in the Department of Civil and Environmental Engineering at the University of California Davis, and is a Graduate Advisor for the Conservation Biology Area of Emphasis in the Graduate Group in Ecology. Trained as a mammalian population biologist, he has been conducting mark-recapture studies on mammals for over 20 years and has numerous publications on the demography and dispersal. He holds all appropriate federal and state permits to conduct the research.

Natalia Belfiore is a PhD Candidate in the Graduate Group in Ecology at UC Davis. She has extensive past experience in field surveys and trapping projects with mammals. In addition, she has experience managing projects and supervising assistants through a variety of research projects and employment opportunities.

g. Biomarker data will be used to assess the effects of several classes of pesticides applied in the Delta on otters. Genetic data will be used to test the predictions of genetic change in populations in response to selective pressure from contaminants. Statistics calculated from genetic differences at a variety of microsatellite loci will be used to test specific hypotheses regarding the effect of selection by pesticides and of genotoxic damage on population-level genetic change. Genetic patterns and biomarker evidence in river otters will then be correlated to spatial maps of land use and pesticide inputs created in the first step, to build a spatial understanding of the impacts of pesticides on otters in the Delta. Data will ultimately be evaluated to address the four questions posed above. In particular, it will be determined if otters are being negatively affected by chronic pesticide exposure, if biomarker surveys may be used to assess these impacts, and if genetic data provide markers of long term vulnerability.

h. Coordination with efforts of local wildlife agencies and conservation groups is expected to be successful, based on preliminary contacts. Key individuals have expressed interest in the outcome of the project and in the top-carnivore approach. The goals of the project as well as the anticipated benefits of understanding the long-term sublethal impacts of chronic pesticide exposure are consistent with each component of the CALFED mission statement. Several of the target habitats and stressors are considered by this study due to its unique integration of ecosystem components and habitat types in the study of one important indicator species.

## PROPOSAL COVER SHEET

**Proposal to:** CALFED Bay-Delta Program Office  
1416 Ninth Street  
Suite 1155  
Sacramento, CA 95814

**Submitting Organization:**  
The Regents of the University of California  
University of California  
Davis, CA 95616

**Title of Proposed Research:** The Genotoxic and Genetic Effects of Pesticides on River  
Otters in the Sacramento-San Joaquin Delta of California

Total Amount Requested	Proposed Duration	Desired Starting Date
\$108,591	Three Years	October 1, 1997

Principal Investigator:	Department:	Phone Number
Michael L. Johnson	CEE	(916) 752-8837

**Checks made payable to:**  
The Regents of the University of California

**Send checks to:**  
University of California  
Davis Campus  
Cashier's Office, 173 Mrak Hall  
Davis, CA 95616

**Send Award Notice to:**  
Office of Research  
410 Mrak Hall  
University of California  
Davis, CA 95616  
(916) 752-2075

### Approvals:

Michael L. Johnson 7/22/97  
Principal Investigator Date

Daniel R. Chay 7/22/97  
Department Chair Date

W. McLoey 7/23  
Dean, College/School Date

\_\_\_\_\_  
Other Endorsement Date

Sandra M. Dowdy  
Official Signing for Organization Date  
Sandra M. Dowdy  
Contracts and Grants Analyst

## II. Title Page

- a. Title: The Genotoxic and Genetic Effects of Pesticides on River Otters in the Sacramento-San Joaquin Delta of California
- b. Dr. Michael Johnson  
Department of Civil and Environmental Engineering  
Everson Hall, Davis California, 95616  
tel: (916) 752-8837  
fax: (916) 2-7872  
e-mail: mbjohnson@ucdavis.edu  
Research Associate, University of California, Davis
- c. State University (State Agency); Tax status: exempt.
- d. Tax identification number: 94-6036494-W
- e. Technical contact person: same as above.  
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- f. Dr. Michael Johnson, Department of Civil and Environmental Engineering  
Natalia M. Belfiore, Graduate student, Graduate Group in Ecology, UC Davis
- g. RFP Project Group Type: Other Services

### III. Project Description

a. **Project description and approach-** The proposed research addresses the effects of pesticides applied within a California ecosystem and attempts to further the understanding of mechanisms and processes that will serve to advance the predictive power of theory to assess ecosystem quality and the vulnerability of sensitive and indicator species. The North American river otter, *Lutra canadensis* (Schreber 1776), was selected as a good animal model to study the effects of contaminants in a riverine habitat because of its position as a top-carnivore in the system. Contaminants present in otter ranges have been suggested as a likely cause of otter population decline (e.g., Mason, 1989; Wren, 1991; Halbrook *et al.*, 1994). The San Francisco Estuary project suggested monitoring the river otter in the Sacramento-San Joaquin Bay-Delta (Delta) as an indicator of contaminant exposure to wildlife (Bailey *et al.*, 1995) but concedes that most funding goes toward research on fish species. The current project includes a biomarker investigation to quantify exposure to and short-term effects as well as genetic investigation to estimate long-term effects of important pesticides on river otters in the Delta.

**Goals** The goals of this research are three-fold:

- 1) to evaluate the effects of pesticides on river otters in the Delta
- 2) to test predictions of the long-term impacts of pesticides on genetic change in river otter populations
- 3) to estimate the relative influence of pesticide contamination in the Delta on river otter decline in California.

The research is designed to test four specific questions. Will biomarker responses among river otters exposed to different concentrations of pesticides vary with dose? Will biomarker responses permit me to link exposure with direct damage to individuals? Will genetic differences, if found, be consistent in river otters across field replicates? And finally, can biomarker responses complement population-genetic patterns when building a spatial understanding of bioavailable contaminants?

The project has four phases. During Phase I, sampling locations are being selected. This involves a preliminary distribution assessment of pesticides and otters. During this phase, agricultural databases and otter and pesticide survey information from local agencies as well as field surveys, are compiled for review to determine patterns of pesticide inputs and otter populations to select experimental and reference locations for comparison. The latter part of Phase I involves the collection of crayfish and possibly otter spraint (fecal) samples from selected locations for chemical analysis to confirm site characterization that was achieved by indirect mapping methods. The confirmation of pesticide residues in these media will closely link otters to exposure.

In Phase II, otter spraints will be systematically collected from sample locations and analyzed by molecular genetic techniques. Microsatellite DNA variation will provide information about the spatial distribution of otters, and will permit analysis of genetic differences among populations that will later be correlated with exposure parameters measured in the other phases.

In Phase III, otters will be trapped for collection of tissue samples for biomarker analysis. Biomarkers of both exposure and genotoxic damage will be measured. In addition, the health of the animals will be assessed, animals will be marked, and released. This is the most labor-intensive and time-extensive portion of the project as trap success for river otters is relatively low.

However, it is also a critical step to linking pesticide exposure to measures of short term damage, and ultimately to genetic change, a measure of long term effect.

Finally, Phase IV involves the laboratory analysis of samples drawn from otters during Phase III. Serum will be analyzed for acetylcholinesterase levels to ascertain exposure to acetylcholinesterase inhibitors, specifically, to organophosphates and carbamates, the most widely applied pesticides in the Delta. DNA strand breaks have been shown in laboratory studies to be a consequence of exposure to these pesticides, and will be assessed in blood as a measure of genotoxic damage. Values derived from this step will be critical during the final data analysis.

b. **Location-** River otters are known to inhabit a wide range of aquatic habitats throughout northern California. Studies of preferred otter habitat maintain that woody riparian vegetation is necessary for river otters. This is predicted in part because otters use abandoned dens built of vegetation by muskrat and beavers and make burrows at the base of trees. However, river otters and otter sign have been recorded in habitats ranging from highly disturbed agricultural sloughs to pristine shaded riparian waterways. Although the exact extent of the current river otter range in the Delta and the valleys of northern California is not known, it is expected, based on recent observations, that otters will be located in sufficient densities in a minimum of four locations, two within the Delta which are subject to high pesticide inputs, and two within the Yolo, American River, Colusa and Feather River/Sutter Basins upstream of the Delta, that will not be subject to heavy pesticide inputs. Preliminary investigations of recently observed otters include locations in Contra Costa, Yolo, San Joaquin, Colusa, Butte, Glenn, and Tehama counties.

c. **Expected benefits-** River otters have been recently observed to inhabit several habitats of interest to CALFED. Otters have been primarily associated with shaded riverine aquatic habitat due to the abundant availability of fish for food, and woody vegetation and complex banks which are used for denning; their decline since earlier trapping eras has in part been attributed to the loss of these habitat features (Stone, 1976). This habitat-type has been severely reduced in recent decades by intensive development and agricultural activity (San Francisco Estuary Project, 1992). Recent observations, however, indicate that otters also inhabit seasonal wetlands, midchannel islands and shoals, and the north Delta agricultural wetlands, all habitats of concern to CALFED. It is not known whether otters merely come to feed in some of these more one-dimensional habitat-areas, or if they are able to den and reproduce in these areas. It is possible that otters have altered their daily dispersal habits because of the loss of preferred riparian woodland. Restoration of each or all of these habitats should include consideration of habitat quality for river otters. In particular, agricultural sloughs that contain high pesticide runoff and still support otter populations are predicted to impart significant long-term risk to otter population survival. The same risks are predicted to apply to other resident piscivores such as raptors, mink and ringtails.

Several of the stressors of significant interest to CALFED are likely to affect river otters. Geomorphic alterations could impose migration barriers and barriers to access to preferred habitats. Similarly, alteration of channel form, including loss of shallow waters, meanders, sidechannels, as well as shaded riparian habitat are predicted to significantly alter the food and denning resources available to otters. Finally, reduction of water quality, in particular due to high agricultural activity, is expected to impose chronic sublethal stress on otter populations. The combination of these factors is likely to cause severe population reduction in future generations.

These could be the result of genotoxic syndromes causing reduced reproductive success enhanced by alterations in spatial distribution and gene flow due to geomorphic change and contaminants.

Estimating the effects of pesticides on river otter population decline in the Delta is important in the context of risk and conservation assessment of pesticides in the Delta ecosystem. This project is the first to study a mammalian carnivore in this important California region. The research and regulatory communities have recently encouraged the investigation of long-term effects of pesticides, and of effects at multiple levels of the ecosystem, such as those that would affect river otters (Joern and Hoagland, 1996; Landis *et al.*, 1996; Maurer and Holt, 1996). Because of its trophic position, the river otter is an important indicator species for understanding and measuring the quality and dynamics of this endangered ecosystem. For example, the river otter diet in the Delta is comprised largely of crayfish which feed in the sediments and are likely, therefore, to impart doses of accumulated pesticide residues (Grenfell, 1974; Halbrook *et al.*, 1994). The long term effects of these interactions may be reflected in patterns of genetic variation in river otters. These patterns provide a better understanding of the population-level interaction between contaminants and ecological factors which is important to a more complete understanding in toxicology (O'Connor, 1996). Understanding of river otter dispersal and range patterns, validated by genetic patterns, will permit a larger scale assessment of the effects of agricultural pesticides on river otters throughout the Delta and further afield.

d. **Background and Justification-** The Delta contains pesticide loads sufficient to be toxic to invertebrates and fishes and these chemicals have been implicated in the decline of other species such as striped bass (Bailey *et al.*, 1995). Because of its trophic position, otters are likely to bioaccumulate pesticides that are consumed or absorbed by species lower in the food chain. Indeed, several studies document heavy metal and organochlorine pesticide accumulation in otter tissues in other locations (e.g., Foley *et al.*, 1987; Halbrook *et al.*, 1994). The same quantities of chemicals were shown to produce harmful effects in otter, and the related species, mink, in laboratory studies (e.g., Wren, 1991). Organochlorine bioaccumulation has also been correlated with low population densities and slow recovery of European river otters (Mason and Macdonald, 1993). Although organochlorines are not currently widely discharged in the Delta, organochlorines and their breakdown-products persist as residues in this region, and other pesticides, including organophosphates and carbamates are applied in high volumes and have the potential to affect river otters.

The California Department of Food and Agriculture indicates a number of organophosphate and carbamate (including thiocarbamate) pesticides, among others, are applied in large quantities, and at concentrations found to be toxic in many organisms (see Bailey *et al.*, 1995). Organophosphates have been shown to be sufficiently persistent to cause toxic effects in some invertebrates (Norberg-King *et al.*, 1991); organophosphates and carbamates have demonstrated adsorption to sediments long enough to be toxic to organisms feeding or living nearby (Domagalski and Kuivila, 1993). An organophosphate was shown to cause cytological alterations in a fish at chronic sublethal doses (Arnold *et al.*, 1996). In addition, a review of the RTECS database shows genotoxic effects of many pesticides applied in the Delta (RTECS, 1996). For example, 28 of the 56 most heavily applied pesticides showed positive genotoxicity in 5 or more tests reported (RTECS, 1996).

Short-term genotoxic damage to individuals by pesticides are predicted to have long-term, indirect effects on populations (Anderson and Wild, 1994; Landis *et al.*, 1996; Shugart and

Theodorakis, 1996; Anderson and Belfiore, submitted). Genotoxic damage is expected to cause population reduction through direct reproductive impairment, or selection against sensitive or for resistant individuals within populations. Mechanistic connections between contaminant exposure and long-term genetic change have been supported by theory and experimental evidence. Changes in population genetic structure have been correlated with resistance in laboratory studies, in the field in pests, and theoretically (e.g., Mouches *et al.*, 1986; Guttman and Dykhuizen, 1994). By a number of mechanisms suggested in the literature, (e.g., gene frequency change, gene duplication, or changes in genetic variability), genetic change in populations may be dramatic in response to contaminant exposure (e.g., Macnair, 1991; Scott, 1995).

The Delta ecosystem has been the focus of a large number of research and regulatory efforts in California due to its critical role in state-wide water disputes, and in the agriculture industry in the region. For example, changes in water flow patterns and concomitant increases in contaminant concentrations have been implicated in damage to fish communities in the Delta (Moyle, 1996). The proposed work on river otters is consistent with the goals of several regional programs. A report prepared for the Bay/Delta oversight council emphasizes that many contaminants reach toxic concentrations throughout the Delta and that while it is not possible to set standards for every affected species, it is critical to consider the most sensitive and indicative species, such as top mammalian carnivores in which "effects of accumulated contaminants will be most apparent (p 103)." The CALFED Bay Delta Program Overview (1996) points repeatedly to the goal of sustaining and restoring ecosystem health, including restoration of conditions to those that permit long-term viability and integrity of key wildlife species at all ecological levels. In its action plan, the San Francisco Estuary Project's Comprehensive Conservation and Management Plan (1994) includes restoration of healthy habitat conditions to the Delta and assurance of the survival of candidate species and species' in decline; the river otter is in decline and was listed as a Category II species (Endangered Species candidate) before the list's dissolution by Congress.

To date, no specific research or protection efforts have been identified to target important indicator species such as the river otter, in spite of discussions of its importance. Biomarkers are currently considered among the most useful techniques to determine exposure to and effects of contaminants on resident populations. In addition, the importance of the use of markers of genetic change in toxicology was emphasized at the Napa Conference on Genetic and Molecular Ecotoxicology (1993) but has only been attempted in field examinations in a few studies (see Anderson and Belfiore, submitted). This research, therefore, could result in the establishment of an important model animal for use in Delta ecotoxicology studies, in advances in the state of the art in genetic ecotoxicology, and in establishment of a strong body of evidence linking conservation biology and applied ecotoxicology.

e. **Scope of work- Preliminary distribution assessment and sampling design:** Existing records and recent sightings of river otters in the Delta are currently being mapped in a Geographical Information System format. These will be overlaid with maps which detail state records of local agricultural crops, vegetation types, and water parameters in the area. The locations of probable current otter populations are being verified at this time using field mapping of vegetation and habitat conditions (e.g., Dubuc *et al.*, 1990), as well observations of otter sign (e.g., latrines, slides) and otters. Several subpopulations of otters exposed to significant pesticide inputs are being selected for comparison within the Delta region. Several reference populations

will be selected from contributing drainages upstream of the Delta in order to make comparisons with exposed Delta populations.

*Exposure assessment and biological measures of exposure:* Crayfish, spraints (otter feces), and fat biopsies (if permitted) from otters at each sample site will be analyzed for the presence of target pesticides to verify exposure levels. Crayfish have been shown to comprise 90% of otter diets in this region in past studies; diet analysis currently underway appears to confirm this. Spraints will be tested for organophosphates and carbamates and will be used to assess exposure if these pesticides are found to be consistently measurable. Otter spraints are easy to collect on stream banks because otters establish latrines used repeatedly by several individuals. These excretions have been documented to indicate consistent levels of persistent lipophilic contaminants relative to body tissues in other studies (Brinck *et al.*, 1978; Larsson and Lindegren, 1987; Mason *et al.*, 1992; Mason and Macdonald, 1994). Fat biopsies will be taken by needle aspiration during Phase III, if permitted by the animal protocol. These will be tested for lipophilic pesticides to assess their utility for exposure assessments. In addition, biochemical markers of exposure, including persistent metabolites or metabolic blood products related to contaminant metabolism, will be measured in spraints, if identified. Access to exposure markers in spraints permits a non-invasive sampling technique that may result in a larger sample size.

Serum samples from otters at each site will be collected and preserved during Phase III. Otters will be live-trapped for blood-sampling using Hancock beaver suitcase traps following the methods of Serfass *et al.* (1996), an approved animal protocol at UC Davis, and a memorandum of understanding with the California Department of Fish and Game. Cholinesterase activity will be assessed in serum using colorimetric techniques (Ellman *et al.*, 1961) as a measure of exposure to anticholinesterases such as organophosphates, carbamates, and some other pesticides (e.g., Zinkl *et al.*, 1981; Khattab *et al.*, 1994). Spectrophotometry equipment necessary for the analyses is available in the laboratory of Dr. Susan Anderson at Lawrence Berkeley National Laboratory (LBNL).

*Biological measures of effect:* Blood samples collected in the field during Phase III will be used to assess genotoxic damage. DNA strand breaks will be quantified in exposed populations, using the alkaline unwinding assay, and compared to background levels of strand breaks in unexposed populations (Shugart *et al.*, 1992). Assays will be performed on blood, and records of body condition, age, and other physiological variables will be maintained to control for these sources of variation (Sugg *et al.*, 1995). Theodorakis *et al.* (1992) and Sugg *et al.* (1995) showed blood to be a suitable tissue for measuring DNA strand breaks. Protocols are currently being developed for this technique in the laboratory at LBNL.

*Measures of genetic change:* In Phase II, DNA will be extracted from spraints collected at each field site for analysis of genetic variation. Polymerase chain reaction (PCR) amplification methods on fecal extractions have been consistently successful at amplifying DNA of sufficient quality to examine microsatellite DNA variation (Hoss *et al.*, 1992; Gerloff *et al.*, 1995; Ernest, pers. comm.). Otter researchers identified in New York, Oregon, Pennsylvania, and Louisiana will provide sufficient paired samples of otter feces and blood for species identity verification. Once otter DNA is identified, primers and PCR conditions will be optimized and specific sequencing and data interpretation protocols for microsatellites will be developed. The application of PCR/ microsatellite methods from fecal samples ensures that adequately large numbers of individuals may be sampled for genetic data.

Microsatellite DNA will be sequenced using an automated sequencer. DNA sequences will be used to compare within-population variation to assess genetic diversity changes among treatments and to eliminate background genetic variation in a search for exposure-related markers of genetic change. All molecular genetic techniques will be performed in the LBNL core facility.

f. **Monitoring and data evaluation-** Data from each phase of this project will provide an in-depth assessment of a different aspect of the sublethal effects of pesticides on river otters. Chemical and biomarker analyses have been selected to estimate key components of the assessment. The project is designed to be an epidemiological approach to correlate exposure to pesticides to damage. The key to good demonstration of correlation is the intensive collection of key measures that attempt to bridge the span between exposure and long-term sublethal effects. This project attempts to thoroughly examine the four correlates: site concentrations, biochemical exposure markers, DNA damage, and genetic change.

Biomarker data will be explicitly used to assess the effects of pesticides on otters exposed to different concentrations of several targeted pesticides applied in the Delta in large concentrations. Genetic data will be used to test the theoretical predictions of genetic change in populations in response to selection for contaminant resistance or against contaminant sensitivity. Statistics calculated from genetic differences at a variety of microsatellite loci will be used to test specific hypotheses regarding the effect of selection by pesticides and of genotoxic damage on population-level genetic change. Genetic patterns and biomarker evidence in river otters, and pesticide concentrations in otter spraints, will then be correlated to spatial maps of land use and pesticide inputs created in the first step, to build a spatial understanding of the impacts of pesticides on otters in the Delta.

g. **Implementability-** No portion of this project involves laws or regulations regarding land use or environmental impacts. No changes to environmental features, such as hydrologic or geomorphic conditions, are involved. Handling of vertebrate animals requires animal protocol approval by the Environmental Health and Safety office of UC Davis. River otters are protected by the regulations of the California Department of Fish and Game (CDFG); fur-trapping is prohibited and trapping for other purposes requires special permission. To obtain the permit, trappers must be certified by the CDFG. Certification requires participation in a CDFG training course that includes wildlife restraint and handling procedures. Natalia Belfiore has completed the wildlife restraint portion of the training at the UC Davis School of Veterinary Medicine and is scheduled to obtain the handling experience required for complete certification. The goals of the project are consistent with a number of State, Federal, and local environmental agencies and advocacy groups. To date, contacts with local groups that have been forged to obtain assistance and access to information and databases have been positive. It is anticipated, based on experience to date, that most of the needed assistance to gain access to reserves and private lands will be obtained.

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**Cost and Schedule to Implement Proposed Project**  
**Project Period: October 1, 1997 to September 30, 2000**

	<u><b>Year One</b></u>	<u><b>Year Two</b></u>	<u><b>Year Three</b></u>	<u><b>Totals</b></u>
PGRE 6 months @ 100%	\$14,336.*	\$15,052.**	\$15,805.*+	\$45,193.
Technical Support	<u>1,190.+</u>	<u>1,249.++</u>	<u>1,312.+*</u>	<u>3,751.</u>
<b>TOTAL SALARIES</b>	<b>\$15,526.</b>	<b>\$16,301.</b>	<b>\$17,117.</b>	<b>\$48,944.</b>
Fringe Benefits: *17.76%, **18.26%, *+18.76%, +23.62%, ++24.13%, +*24.62%	<u>2,827.</u>	<u>3,049.</u>	<u>3,287.</u>	<u>9,163.</u>
<b>TOTAL PERSONNEL</b>	<b>\$18,353.</b>	<b>\$19,350.</b>	<b>\$20,404.</b>	<b>\$58,107.</b>
Equipment: Outboard Motor	1,600.	0.	0.	1,600.
Supplies	7,750.	1,250.	1,250.	10,250.
Travel	<u>3,670.</u>	<u>780.</u>	<u>780.</u>	<u>5,230.</u>
<b>TOTAL DIRECT COST</b>	<b>\$31,373.</b>	<b>\$21,380.</b>	<b>\$22,434.</b>	<b>\$75,187.</b>
Indirect Costs @ 44.5% Year 1 and 46% Years 2 & 3 (Total Direct Costs less Equipment and Student Fees)	<u>13,249.</u>	<u>9,835.</u>	<u>10,320.</u>	<u>33,404.</u>
<b>TOTAL COSTS</b>	<b>\$44,622.</b>	<b>\$31,215.</b>	<b>\$32,754.</b>	<b>\$108,591.</b>

**NOTE:** Personnel and fees increased by 5% in the second and third years.

#### **IV. Costs and Schedule**

**a. Budget Costs-** There are four main components to the project. The first phase (Phase I) is the site characterization and sampling design phase. **Phase I** is currently underway and involves the compilation of state and research databases of aquatic toxicity data from the Delta and vicinity. Mapping and county record databases of agricultural land use and kilograms per year of pesticide applications are being overlaid onto maps of the region to permit systematic spatial and temporal prediction of pesticide inputs. Finally, records of otter distributions in the area are being combined with recent sightings of otters and otter signs (latrines, slides, dens) to determine where otter populations may be sampled which are subject to high and low pesticide inputs. Actual pesticide residues at the sites will be confirmed by chemical analysis of crayfish and possibly of fat biopsies taken from otters in Phase III. Phase I is currently being funded by an American Wildlife Research Foundation grant, an Association of Women in Science award, and a Jastro Shields Research Scholarship grant to Natalia Belfiore in 1996 and 1997. Analytical chemistry will be funded by the supply funds available to Natalia Belfiore from the UC Toxic Substances Research and Training Program fellowship. No funds are being requested for this phase from CALFED.

**Phase II** of the project is the genetic analysis of microsatellite DNA extracted and amplified from otter spraints. This phase involves collaboration with researchers in other facilities who will provide paired fecal and tissue samples for method development. This stage will be followed by collection of otter spraints from selected field locations for microsatellite analysis. Part of this phase is being supported by the 1997 Jastro Shields Research Scholarship to Natalia Belfiore. Part of this phase is supported by the availability of the Lawrence Berkeley National Laboratory Core Molecular Biology Facility through the collaboration of Dr. Susan Anderson. No funds are being requested for this phase from CALFED.

Funds are requested for **Phase III**, the field component of the project. The field component comprises regular trapping for river otters in the Delta. Trapping will be performed under the supervision of Natalia Belfiore who will be fully certified and permitted by the California Department of Fish and Game and following an animal protocol approved by UC Davis. Hancock beaver suitcase traps will be set at each of the four sample locations following protocols recommended by river otter trapping and reintroduction programs in Idaho, Pennsylvania and New York. Traps will be baited and set in the afternoons. It is anticipated that 30 to 40 traps will be set per night in one sampling location. Traps will be checked daily in the mornings for otters. Details of setting, placing, and monitoring traps will be modified as necessary throughout the trapping program. Trapping will proceed in each location for approximately two weeks, and then moved to the next location.

Every otter that is caught in a trap will be processed according to the protocol established with the animal care committee at UC Davis. Animals will be transferred to handling bags and sedated with a ketamine-medetomidine combination. Anesthesia permits lowered trauma during work-up, as well as increased safety for the handlers. Ketamine-medetomidine persists for approximately 25 minutes, and is then reversed by atipamezole. Reversal assures shorter recovery times, reducing the potential for post-anesthetic complications. During the period of sedation, blood will be drawn for biomarker and genetic analysis. Basic health examinations will be performed. Otters will be ear-tagged for future identification. Atipamezole will be administered and otters released at the same location where they were trapped.

If an otter is found injured, or assessed to be in poor health, measures will be taken to ensure proper care is provided, according to the animal protocol procedures approved by the UC

Davis committee and the California Department of Fish and Game. Animals will be treated and released on site by a licenced veterinarian, or will be taken in approved carriers to a pre-established facility for care and recovery.

Phase III requires full time labor of at least one trained researcher during the years that trapping will proceed. Natalia Belfiore is supported for the 1997-1998 and 1998-1999 academic years through a UCTSRTP fellowship. In addition, field assistance will be required during much of the trapping program in order to have the necessary manual and logistic support to put out traps, operate the boat, and process animals. This proposal requests an assistant at the level of PGRI for 50% time for three years (exact figures are shown on Table 1). In addition, funds are requested for the purchase of a 10 HP outboard motor (\$1600) to be used on a small boat available on loan from Dr. Susan Anderson. The vehicle for transport of the boat and supplies will be the personal vehicle of Natalia Belfiore. Travel funds are requested to defray some of the costs of the use of this vehicle (\$500 per year). Three traps are available on loan from UC Davis, and funds are available through the grants mentioned above to Natalia Belfiore for the purchase of an additional 4 traps. Funds are requested at this time for the purchase of 30 additional traps (\$250 each = \$7500). Funds are also requested for an additional 4 traps (\$1000) to be purchased during the subsequent years of the project to replace damaged or lost traps from the first year of the project. Finally, funds are requested for the purchase of anesthetic drugs and supplies for the processing of animals upon capture (\$250 per year). If funding is not secured from CALFED for Phase III, trapping will proceed with the occasional volunteer assistance of undergraduate students. Equipment and supply funds will be requested from other sources of small grants.

Phase IV is the analysis of samples and data taken from river otters when trapped. This requires the use of a laboratory equipped with several items necessary to analyze tissues for DNA strand breaks, and acetylcholinesterase activity. Much of this equipment has already been identified in laboratories of UC Davis researchers and will be available for collaborative use for this project. Funding for the personnel to analyze the samples is secured for 2 years for Natalia Belfiore through the UCTSRTP fellowship. No funding is requested for this phase from CALFED.

**b. *Schedule Milestones-*** Phase I is currently underway and is anticipated to be completed by the end of October, 1997. Phase II will begin during the Fall of 1997, as samples arrive from collaborating facilities. Phase III will begin as soon as funds are available. Trapping protocol is currently being designed and initial trapping trials will begin before the start of the rainy season in 1997 or as soon as is feasible in the spring of 1998. Trapping will proceed full time throughout the dry season (anticipated through at least September) for each year of the project. When trapping is not underway, implementation of the laboratory phases of the project will proceed. The trap success for river otters is reasonably low using non-harmful methods (such as the suitcase traps) so efforts will have to be high to ensure sufficient samples to address the questions. Payments by CALFED would be related to the timing of the funds, rather than the phase, since the phase for which funding is requested is ongoing. It would be necessary to obtain funds at the start of each research year to ensure that funds would be available to hire an assistant and purchase necessary equipment.

**c. *Third Party Impacts-*** This project is anticipated to have no immediate third party impacts. No action levels and no direct mitigation procedures are proposed.

## V. Applicant Qualifications

Project funds and implementation are supervised by Dr. Michael Johnson. Implementation of the field research project and all permits are the responsibility of Natalia Belfiore. Hiring and supervision of the PGRI for whom funds are requested in this proposal are the ultimate responsibility of Dr. Michael Johnson, to be assisted by Natalia Belfiore. Dr. Michael Johnson is an Associate Research Engineer in the Department of Civil and Environmental Engineering at the University of California Davis, and is a Graduate Advisor for the Conservation Biology Area of Emphasis in the Graduate Group in Ecology. Trained as a mammalian population biologist, he has been conducting mark-recapture studies on mammals for over 20 years and has numerous publications on the demography and dispersal. He holds all appropriate federal and state permits to conduct the research

Natalia Belfiore is a PhD Candidate who has just completed her second year in the Graduate Group in Ecology, UC Davis. She completed her MS in Zoology at the University of Florida in 1991, and her BA in Environmental Studies, Biological Basis of Behavior, and French at the University of Pennsylvania in 1984. She has completed field and laboratory research projects in a number of locations around the world and involving many vertebrate species. Field research involving mammals includes the design and implementation of a small mammal trapping program in Washington DC, trapping and handling of raccoons and opossums in Washington DC, tracking and field study of red spider monkeys in Costa Rica, and the design of capture and sampling of nutria in Louisiana and Argentina. She has worked extensively in two molecular genetic projects, for two years in the Genetics Laboratory of the National Zoological Park and for her masters research project at the University of Florida. She also worked for two years for an environmental consulting company in Alameda California and has experience interpreting environmental regulations and coordinating efforts of multiple agencies and interests.

In recent years, she has been involved in data collection using genetic biomarkers and assessment of biomarker techniques as they have been used in field applications in the laboratory of Dr. Susan Anderson (LBNL). In addition, training in animal handling and issues of wildlife epidemiology has been ongoing by Drs. Walter Boyce, David Jessup, Jonna Mazet (DVM, UC Davis). Direct training in handling wild river otters is currently being negotiated with Dr. George Kollias, DVM, at Cornell University. Natalia was selected to participate in the National Opportunities for Animal Health (National Cancer Institute and National Zoological Park), training course in conservation genetics, scheduled for August of 1997. She is a co-author on one publication, and has four manuscripts in progress.

## **VI. Compliance with Standard Terms and Conditions**

Standard terms and conditions of this contract are those that apply to state agencies as shown in Table D-1 of the RFP. These do not require any paperwork until the final contract is in place. The standard University of California Office of Research Data Sheet has been completed and includes approval for submittal of this proposal by the Dean of the College of Engineering, and the UC Davis Office of Research. This approval includes agreement by Dr. Michael Johnson to the standard terms and conditions of the UC Davis OR data sheet. These forms are attached.

## NONDISCRIMINATION COMPLIANCE STATEMENT

COMPANY NAME

THE REGENTS OF THE UNIVERSITY  
OF CALIFORNIA

The company named above (hereinafter referred to as "prospective contractor") hereby certifies, unless specifically exempted, compliance with Government Code Section 12990 (a-f) and California Code of Regulations, Title 2, Division 4, Chapter 5 in matters relating to reporting requirements and the development, implementation and maintenance of a Nondiscrimination Program. Prospective contractor agrees not to unlawfully discriminate, harass or allow harassment against any employee or applicant for employment because of sex, race, color, ancestry, religious creed, national origin, disability (including HIV and AIDS), medical condition (cancer), age, marital status, denial of family and medical care leave and denial of pregnancy disability leave.

## CERTIFICATION

*I, the official named below, hereby swear that I am duly authorized to legally bind the prospective contractor to the above described certification. I am fully aware that this certification, executed on the date and in the county below, is made under penalty of perjury under the laws of the State of California.*

OFFICIAL'S NAME

Sandra M. Dowdy  
Contracts and Grants Analyst

DATE EXECUTED

JUL 25 1997

EXECUTED IN THE COUNTY OF

Yolo

PROSPECTIVE CONTRACTOR'S SIGNATURE

PROSPECTIVE CONTRACTOR'S TITLE

PROSPECTIVE CONTRACTOR'S LEGAL BUSINESS NAME

THE REGENTS OF THE UNIVERSITY  
OF CALIFORNIA